

Use of Reference Samples in Evaluating Water Laboratories

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UNDER REGULATIONS adopted in 1951 (1) water laboratories in California must be approved by the California State Department of Public Health if their findings are to be accepted by a health jurisdiction. Development of the regulations and proposed methods of operation under them have been described by Hartmann (2). As was observed, satisfactory operation of an approval program requires adequate methods for assessing work quality both prior to and after granting approval. Use of reference, or check, samples has proved the single best means of meeting this need. Since 1952, reference samples, either for chemical or bacteriological analysis, have been distributed to these laboratories, which now number 107. Results obtained from analysis of these samples have formed the basis for additional control measures.

The Analytical Reference Service of the Public Health Service has more recently developed a similar reference program for State laboratories (3). The national program, however, is voluntary, whereas in California participation is a condition for obtaining and maintaining an approved status. Also, the national program has as a major goal the evaluation of methods, while the California program is principally for control of work quality.

As an example of how the California program operates and to show generally how reference samples may be used, a detailed description is

presented of one chemical reference sample, distributed in January 1960 to the 63 laboratories approved for chemical analysis. This report summarizes and evaluates the results obtained by the participants.

Preparation of Sample

Ideally a reference sample should contain known amounts of the constituents to be sought (3). Unfortunately, because of the large volume required, it was impractical to prepare a standard solution. Instead, the laboratories were sent replicate 1-gallon samples of water bottled in clean, dry containers at a water treatment plant handling surface water. An instruction sheet, which was also the report form, accompanied each sample. Analyses for calcium, magnesium, sodium, potassium, alkalinity, chloride, and sulfate were required, but not all laboratories made all analyses. The laboratories were asked to treat the reference sample as they would routine samples except each analysis was to be made in duplicate. Details of the methods used, the initials of the analyst, and the laboratory worksheet were also requested. In the sanitation and radiation laboratory of the State health department each of seven chemists individually analyzed the reference sample in an attempt to provide basic information on its composition and the variability of results.

Results

A summary of the results obtained is shown in table 1. In calculating the mean and standard deviation for the participating laboratories, a two-step procedure was used (4). First, the averages of all paired values were used to calculate the grand mean and standard deviation

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for each constituent. Second, all values differing from the grand mean by more than ± 2 standard deviations were excluded, and the mean and standard deviation were recalculated. This procedure is open to statistical question, but in practice it serves to eliminate an undue influence of gross and obvious errors such as dilution errors. The coefficient of variation expresses the standard deviation as a percentage of the mean.

Data on analytic methods used by the laboratories are summarized in table 2. Here, the mean and standard deviation were calculated conventionally; therefore, the data in tables 1 and 2 are not directly comparable. The data in table 2 are useful, however, in comparing different methods for the same constituent. For all constituents except alkalinity one method was definitely the predominant choice among the laboratories. For calcium and magnesium, it was EDTA titration; for sodium and potassium, flame spectrophotometry; for chloride, Mohr titration; and for sulfate, the gravimetric procedure. In determination of alkalinity the number of laboratories using the pH meter and the number using methyl orange indicator were about equal. These generalized observations closely parallel those obtained by the PHS Analytical Reference Service (3). With few exceptions the methods used were in accordance with Standard Methods (5).

Discussion

Inspection of the data in table 1 shows that results obtained in the State health department

laboratory were less variable than those obtained by the participating laboratories. In the State health department laboratory there were only two major sources of variation: (a) differences between replicate samples and (b) differences between analysts in the same laboratory using the same methods. For the other laboratories the sources of variation were more numerous and possibly more significant. They were: (a) differences between replicate samples, (b) differences between laboratories, (c) differences between analysts, (d) differences between methods. Because of these differences, results of each laboratory were compared with those obtained by all the participating laboratories rather than with the results of the State health department laboratory. Therefore, the means and standard deviations for the approved laboratories shown in table 1, calculated by the two-step procedure, do not include results of the State health department laboratory.

Results of the duplicate analyses made by each laboratory were in most instances identical or very similar. Therefore, no statistical analysis was made of the paired values. Judging by the initials of the analysts given on the reports, the duplicate analyses were generally made by one chemist, although in a number of laboratories two or more chemists participated. Laboratory worksheets submitted by the laboratories proved quite useful. First, they permitted an examination of analytic details and recordkeeping procedures, and second, all results which were grossly deviant from others could be checked. In a number of re-

Table 1. Summary of results obtained on analysis of reference sample by the sanitation and radiation laboratory of the California State Department of Public Health and approved water laboratories in the State

Constituent	Sanitation and radiation laboratory			Approved laboratories		
	Mean (mg./l.)	Standard deviation (mg./l.)	Coefficient of variation (percent)	Mean (mg./l.)	Standard deviation (mg./l.)	Coefficient of variation (percent)
Calcium.....	46.0	0.675	1.47	46.5	1.87	4.02
Magnesium.....	21.3	.499	2.35	21.2	2.54	12.0
Sodium.....	75.0	1.52	2.03	75.9	4.25	5.60
Potassium.....	3.8	.180	4.76	3.5	.67	19.1
Alkalinity.....	84.1	1.80	2.14	89.5	3.82	4.26
Chloride.....	114.1	1.33	1.16	114.6	3.24	2.83
Sulfate.....	110.5	2.03	1.84	109.8	5.44	4.96

ports significant arithmetic errors were found and corrected.

For evaluating the performance of the participating laboratories the following arbitrary criteria were used: (a) results falling between the mean and ± 1 standard deviation were acceptable; (b) results between ± 1 and ± 2 standard deviations from the mean were acceptable but questionable; and (c) results outside the limits of ± 2 standard deviations were unacceptable. A summary of performance by these criteria is shown in table 3. Of the 63 participating laboratories, 1 laboratory produced unacceptable results for 6 determinations,

1 for 4 determinations, 1 for 3 determinations, 7 for 2 determinations, and 19 for 1 determination. Thus 29 laboratories, or 46 percent, produced unacceptable results for one or more constituents. These laboratories will receive continued close scrutiny.

On a percentage basis the mean of the results on calcium reported by the participating laboratories was essentially equal to the mean of the results obtained in our laboratory (46.5 v. 46.0 mg./l.). The frequency distribution of high and low values was about equal, although the number of unacceptable results, eight, was high. Surprisingly, the results obtained by EDTA

Table 2. Summary of methods used in analyzing a reference sample by approved water laboratories in California

Constituent and method ¹	Laboratories using method		Mean (mg./l.)	Standard deviation (mg./l.)	Coefficient of variation (percent)
	Number	Percent			
Calcium:					
EDTA.....	45	75	46.2	2.10	4.54
KMnO ₄	9	15	46.4	3.39	7.30
Gravimetric (CaO).....	4	7			
Flame*.....	2	3			
Magnesium:					
EDTA.....	40	67	21.9	5.22	23.9
Gravimetric (Mg ₂ P ₂ O ₇).....	9	15	21.8	1.44	6.61
Colorimetric (titan* or brilliant yellow).....	6	10	20.2	1.35	6.68
Flame*.....	2	3			
Unknown.....	3	5			
Sodium:					
Flame.....	40	74	74.4	8.38	11.3
Gravimetric.....	8	15	77.0	1.73	2.25
Calculated.....	4	7			
Spectrographic*.....	1	2			
Unknown.....	1	2			
Potassium:					
Flame.....	42	79	3.6	0.61	17.1
Cobaltinitrite.....	8	15	2.6	2.58	98.7
Spectrographic*.....	1	2			
Gravimetric*.....	1	2			
Unknown.....	1	2			
Alkalinity:					
Electrometric.....	29	45	89.4	3.07	3.44
Methyl orange.....	22	34	87.7	8.98	10.2
Mixed indicator.....	5	8	89.5	3.84	4.29
Methyl purple*.....	4	5	85.6	2.10	2.45
Unknown.....	5	8			
Chloride:					
Mohr.....	43	69	116.3	5.55	4.77
Mercuric nitrate.....	15	24	108.2	5.40	4.99
Electrometric*.....	3	5			
Unknown.....	1	2			
Sulfate:					
Gravimetric.....	44	75	110.6	6.39	5.78
Titrimetric.....	7	12	110.2	5.38	4.88
Turbidimetric.....	6	10	114.5	16.2	14.2
Unknown.....	2	3			

¹ All methods except those marked with an asterisk are from Standard Methods (5).

titration with various indicators were less variable than those obtained by the combined gravimetric-KMnO₄ standard titration method, but the mean values were almost identical.

For magnesium the mean obtained by the participants was essentially the same as ours (21.2 v. 21.3 mg./l.). Grossly deviant high values appeared more frequently than comparable low values. The overall variability of results was unexpectedly high, but the number of variant laboratories was among the lowest (table 3). From table 2 it can be seen that this high variability is due principally to variations in results of the EDTA titration. Explanations for the difficulty are not obvious, but it may be related to choice of indicator.

Table 3. Number of approved water laboratories in California with questionable or unacceptable results on analysis of reference sample

Constituent	Questionable results ¹	Unacceptable results ²
Calcium.....	16	8
Magnesium.....	8	5
Sodium.....	16	5
Potassium.....	15	8
Alkalinity.....	16	5
Chloride.....	18	7
Sulfate.....	16	8

¹ Between ± 1 and ± 2 standard deviations from the mean.

² Greater than ± 2 standard deviations from the mean.

The mean of the values obtained for sodium by the participants was almost identical with ours (75.9 v. 75.0 mg./l.). High and low values seemed to be equally distributed although all unacceptable values were low ones. Results obtained by the gravimetric procedure were less variable but higher than those of spectrophotometry.

The mean for potassium obtained by the participants was significantly lower than ours (3.5 v. 3.8 mg./l.). More unacceptable low results were produced than unacceptable high results. Most of the unacceptable results were obtained by means of a cobaltinitrite procedure. Unfamiliarity with the methods of determining potassium, a determination which is not often made, probably accounts for these results.

For alkalinity the mean obtained by the par-

ticipants was significantly higher than ours (89.5 v. 84.1 mg./l.). This discrepancy may be attributed to the pH selected as the end point of the titration. Dong and associates (6) have shown the errors resulting from selection of an end point which does not correspond to the equivalence point of the bicarbonate titration. We used a pH of 4.8. If, in the absence of any data, it is assumed that the pH used by the participants was that of methyl orange, 4.3, and the correction suggested by Dong and his co-workers is applied, then the average alkalinity for the participants becomes 83.7 mg./l. It may be concluded that many of the apparent discrepancies in the results are due to end point selection rather than real errors. The average results obtained with methyl purple (table 2), which changes color at pH 4.8, tends to support this belief. From table 2 it may be further concluded that the use of methyl orange as the end point indicator is generally less satisfactory than the use of a pH meter irrespective of the pH selected for the end point.

The two means for chloride were essentially identical (114.6 v. 114.1 mg./l.). The frequency of high and low values appears normal. Both the Mohr and mercuric nitrate methods were equally variable, but the mercuric nitrate titration gave lower results, on the average, than the Mohr titration (table 2).

For sulfate the two means were again essentially identical (109.8 v. 110.5 mg./l.). The gravimetric procedure yielded higher and considerably less variable results than the turbidimetric methods.

To check independently the correctness of any analysis, the simple anion-cation balances method of Greenberg and Navone (7) can be used. Although this was not done for each laboratory it was done for the averages of all the laboratories. The average sum of the cations (7.45 meq./l.) compares favorably with the anion sum (7.31 meq./l.), indicating overall acceptability of results.

This reference sample showed that performance by a small number of laboratories was, on the whole, unacceptable. Further, it demonstrated that performance of a larger number of laboratories was better, but occasionally unacceptable. With this information, the State health department laboratory was able to insti-

tute a followup program to improve those laboratories needing it.

Initially, we wrote a letter to each laboratory pointing out its deficiencies, if any, and urging immediate corrective action. Then we visited these laboratories to discuss their deficiencies, review their methods, and generally survey their operations. Repeat reference samples will be submitted to the laboratories to measure again the quality of performance.

This followup procedure has been used during the past year with all laboratories first applying for approval. Several of the laboratories initially denied approval were later approved as a result of significant improvements in their work.

Summary

To illustrate use of reference samples in California in assessing quality of work done by approved water laboratories, the story of one chemical reference sample distributed to 63 laboratories is presented. Calcium, magnesium, sodium, potassium, alkalinity, chloride, and sulfate determinations were required. Statistical analysis of the data permitted an evaluation of the quality of performance as well as yielding comparative information on analytic procedures.

Of a total of 63 participating laboratories 29 returned unacceptable results for one or more of the seven constituents. These findings were used as a basis for a program of closer supervision of the laboratories, with, it is hoped, a significant improvement in the quality of water laboratory work in California.

REFERENCES

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